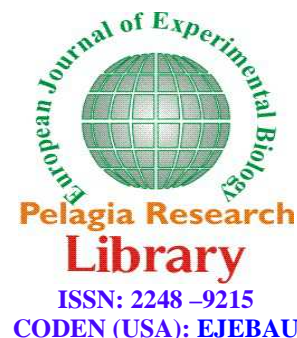




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Biodegradation of Carbofuran using bacteria from pretreated plantain field

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ABSTRACT

Unscientific and wide spread use of pesticides resulted in pollution of whole environment leads to serious health hazard and some are persist in soil and ground water. Pesticides will encounter the degradation when applied to soil by microbial, chemical and photo degradation. Microbial degradation is the breakdown of pesticides by fungi, bacteria and other microorganisms that use pesticide as a nutrient source. Today more than 10% of the pesticides in market are carcinogens. Around 1000 pesticide formulations using throughout the world .In the present study, the Carbofuran(CF) degradation was checked with 3 Bacillus sp, Micrococci sp, and Klebsiella sp were isolated from Carbofuran pre treated plantain field from different parts of Ernakulam district. The bacteria were characterized by biochemical reactions and were identified based on Bergey's Manual determinative bacteriology. Different concentration of Carbofuran-5gm, 10gm, 15gm, 20gm, 25gm were treated with each organism. All the test organisms showed the degradation of Carbofuran in all concentrations. Bacillus sp (E3) shows comparatively more degradation of Carbofuran .Therefore these 5 bacterial strains can be used for bioremediation of Carbofuran polluted areas.

Keywords: Carbofuran, biodegradation, furudan, pesticide, pollution.

INTRODUCTION

In 2011 the State Govt: of Kerala ban almost 8 powerful pesticides including Furudan in the state, but it is used in some areas and the some of the effect remains insist in contaminated areas . "Red category pesticides are available at the shops in Tamil Nadu-Kerala border areas" Reported in 'THE HINDU' in 2013 September .Pesticides are substances used to kill or control pests including insecticides, herbicides, fungicides, rodenticides, molluscides and others. Around 1000 pesticide formulations are used through out the world today. Most Carbofuran is applied by commercial applicators using closed systems with engineered controls so there is no exposure to it in preparation. Since its toxic effects are due to its activity as a cholinesterase inhibitor it is considered a neurotoxic pesticide. Carbofuran is also a powerful endocrine disruptor that can cause transient alterations in the concentration of many hormones in animals and humans even at extremely low doses. These alterations may consequently lead to serious reproductive problems following repeated exposure[1,2]. According to the World Health Organization (WHO), over half a million people are poisoned in each year by the pesticides and five thousand of the victims die[3]. Carbofuran (CF) is an insecticide of N-methyl carbamates family and a nematicide, first brought on to the market in 1965. Carbofuran used extensively in agriculture fields, which cause contamination of soil and ground water system thus polluting the environment and persist for long term. Carbofuran has a high potential for ground water contamination and has been detected in surface waters also. Soil microbes may also be able to degrade Carbofuran.

This work is the preliminary in-vitro study on degradation of the carbofuran by bacteria isolated from the soil which polluted with carbofuran. 5 concentration such as 3%, 2.5%, 2%, 1.5%, 1%, and 0.5% of Carbofuran and 5 bacterial strains were used in this study. Compare degradation ability of all organisms and *Bacillus* sp (E3) shows maximum degradation than other organisms.

MATERIALS AND METHODS

Sample collection and isolation of organisms

Carbofuran purchased from rural area market – 3%- was used in the present study. Soil samples collected from the Carbofuran pre-treated plantain field of three different areas viz. Perumbavoor, Kalady, and Muvattupuzha of Ernakulam district in Kerala. A minimum of five samples were collected from each field, 5cm beneath the surface of soil under aseptic conditions in sterile containers and brought to the lab for further studies. Serial dilution agar plating method was followed for the isolation of micro organisms. Selected Carbofuran degrading organisms were characterized by physiological, morphological and biochemical characters.

Carbofuran degradation study by bacteria

To study the levels of degradation of Carbofuran by the isolates under experimental condition using, Carbofuran as the sole source of carbon. 3%, 2.5%, 2%, 1.5%, 1%, and 0.5% of Carbofuran taken in 6 sets of five flasks 5 for each organisms and 1 set for control containing minimal media (Minimal Media- K_2HPO_4 .2.4g, KH_2PO_4 .1.2g, NH_4NO_3 .1g, $MgSO_4 \cdot 7H_2O$.0.2, $CaCl_2 \cdot 2H_2O$.25mg and $2Fe_2(SO_4)_3$.8mg) used for treatment . Flasks were inoculated with the test organisms respectively and incubate all the flasks at room temperature in a rotary shaker at 120rpm. Degradation ability of all organisms were checked by taking optical density at 600nm in 12hr intervals up to 96hrs and color change from dark violet to pale color for 7days.

RESULTS AND DISCUSSION

The test organisms used in this study were isolated from carbofuran pre-treated plantain field. Selected organisms were identified as 3 *Bacillus* sp, *Micrococcus* sp and *Klebsiella* sp based on Morphological, physiological and biochemical characteristics (Table-1). Biodegradation studies using these test organisms were determined by checking the OD of each flask containing 3%, 2.5%, 2%, 1.5%, 1%, and 0.5% of Carbofuran with respective organisms (Figure-1) and color change from deep violet to pale color in 12hr intervals. All organisms shows degradation ability and violet color of media treated with all organisms decreased well.

Numerous studies have been described for the degradation of Carbofuran in soil[4,5]. 3 *Bacillus* sp, *Micrococci* sp and *Klebsiella* sp isolated from Pre treated plantain field and identified based on Bergey's Manual of determinative Bacteriology .Morphological, physiological and biochemical characteristics of the colony isolated from the pre treated plantain field has revealed by the presence of *Bacillus* sp on the basis of classification schemes published by Bergey's Manual of determinative Bacteriology[6]. The bacteria used Carbofuran as the sole source of carbon and nitrogen for growth to form Carbofuran phenol. The Carbofuran degradation ability was checked and compared based on the color change from deep violet to light violet and also by OD value in 12 hrs intervals. All 5 test organisms shows degradation ability in all 5 concentrations of carbofuran. A number of isolates capable of carrying out some form of carbofuran degradation have been isolated from Soils and bacteria including *Pseudomonas*, *Flavobacterium*, *Arthrobactr* and *Sphingomonas*. [7,8, 9,10]. Higher degradation was obtained in 0.5% Of carbofuran in 96hrs for all organisms and 90% of deep violet color changed to pale violet color up to 7th day. The increase in OD was proportional to the decrease in the violet color. From the OD and Color change, more degradation ability was obtained for *Bacillus* sp (E3) than the other test organisms studied. *Gliocladium* has highest ability to degrade carbofuran than the other organisms were studied such as 28bacteria and another one fungus[11].

Table-1.Morphological and Biochemical analysis

No.	Biochemical test	E1	E2	E3	E4	E5
1	Grams reaction	+	+	+	+	-
2	Shape	Rod	Rod	Rod	Cocci	Rod
3	Colony Morphology	Large round	Large irregular	Medium round	Small round	Mucoid
4	Aerobic growth	+	+	+	+	+
5	Growth at 55°C	-	+	-	-	-
6	Mac Conkey growth	+, NLF	+, NLF	+, NF	+, NLF	+, LF
7	Motility	+	+	+	-	-
8	Endospore	+	+	+	-	-
9	Capsule	+	+	+	+	+
10	Starch hydrolysis	+	+	+	+	-
11	Gelatin hydrolysis	-	-	-	+	-
12	Glucose fermentation	-	-	-	+	-
13	Lactose fermentation	-	-	-	-	+
14	Sucrose fermentation	-	-	-	-	-
15	Mannitol fermentation	-	-	-	-	-
16	H ₂ S production	-	-	+	-	-
17	Indole test	+	-	-	+	-
18	MR test	+	+	-	-	+
19	VP test	-	-	+	-	-
20	Citrate test	-	-	-	-	+
21	Catalase test	+	+	+	+	-
22	Oxidase test	+	-	-	±	+
23	Urease test	+	+	+	-	+
24	6.5% NaCl	-	-	-	+	-
25	Haemolysis	-	-	-	-	-
26	Pigmentation	Pale white	Pale white	Pale white	Yellow	Pale white
Identify (genus)		<i>Bacillus sp</i>		<i>Bacillus sp</i>	<i>Micrococci sp</i>	<i>Klebsiella sp</i>

Figure-1 -Degradation studies of Carbofuran by test organisms

Figure 1.a. E1

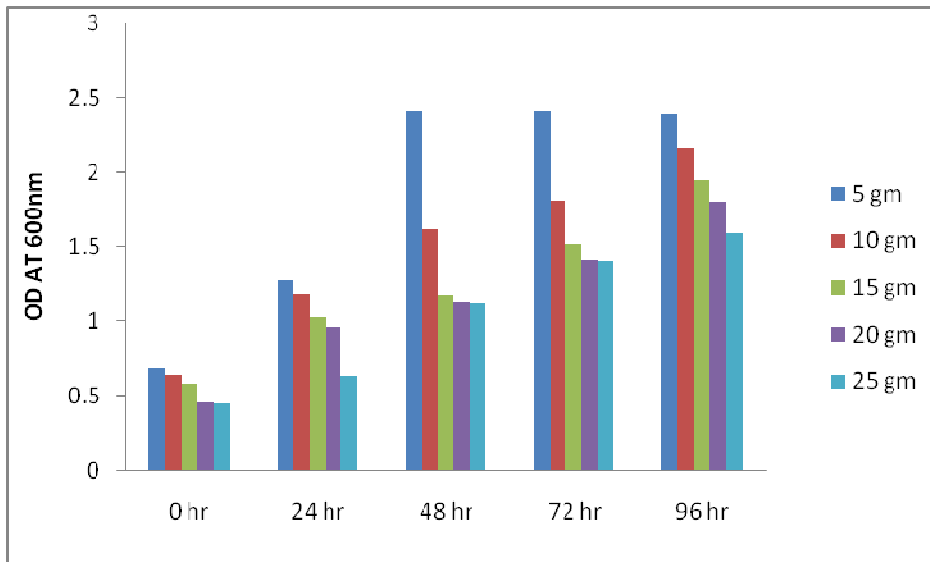


Figure 1.b. E2

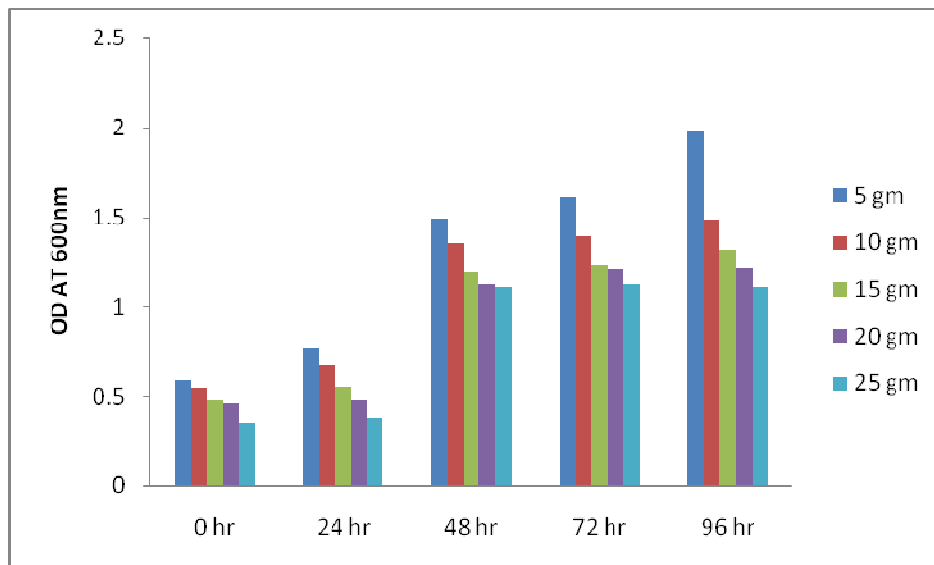


Figure 1.c. E3

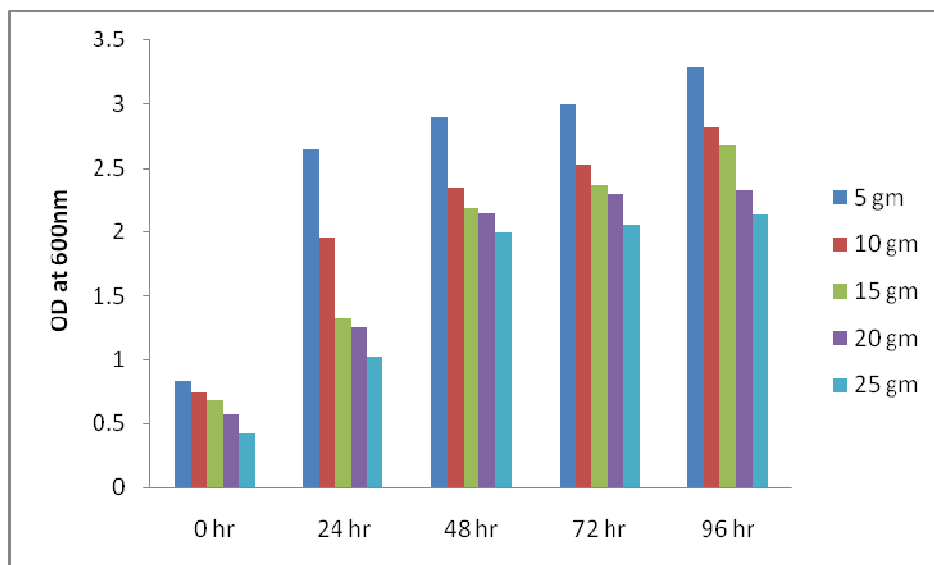


Figure 1.d. E4

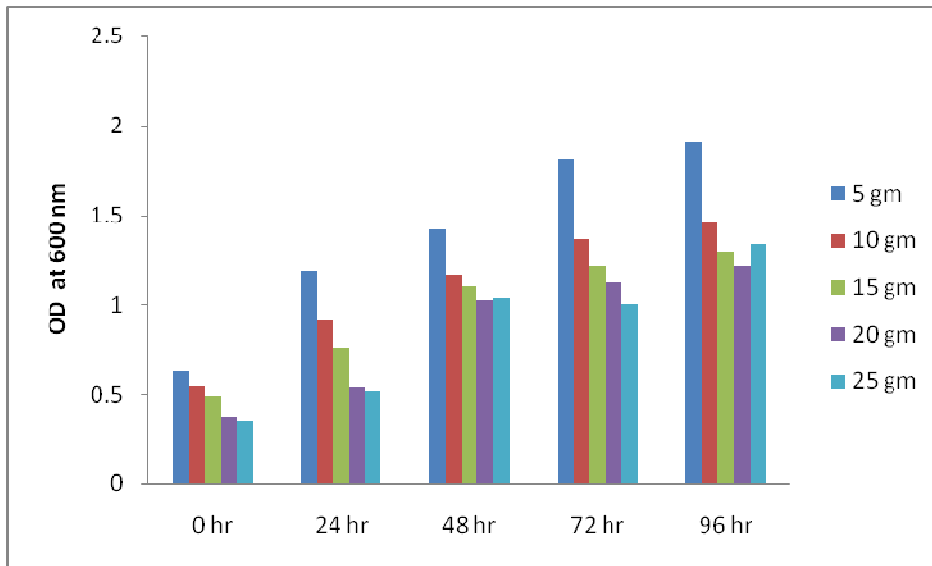
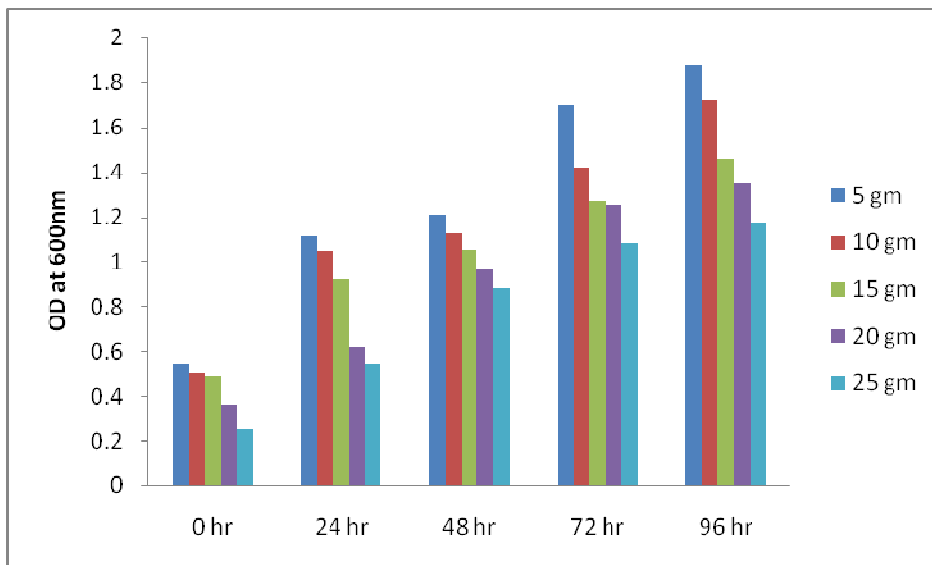


Figure 1.e. E5



CONCLUSION

Here need a great attention and awareness about the use of pesticides; it leads to dangerous toxicity in nature and other living organisms. The present study trying to overcome the effects of carbofuran by bacteria as a bio remedial source. Results shows that, all test organisms *Bacillus* sp, *Micrococci* sp, *Klebsiella* sp has able to degrade carbofuran otherwise they use carbofuran as source of energy to grow and degrade. When comparing the degradation ability of test organisms E3 has the higher activity. Even now the strong pesticides being used in agriculture field and the pollutants must present in the areas where already polluted. Test organisms are able to decrease the Carbofuran hazards of contaminated area. Therefore these 5 bacteria can be used for bioremediation purpose of CF pollution in agriculture field also to overcome the problems of pesticide pollution in environment.

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